Serological tests for COVID-19

Serological assays for SARS-CoV-2 present challenges and opportunities

Timely, scalable and accurate diagnostic testing is crucial in the prevention and control of the coronavirus disease 2019 (COVID-19) pandemic.[1] With limited treatment options and no available vaccine, the accurate and timely identification of infectious patients with COVID-19 is instrumental to the public health outbreak response. Isolation of patients with COVID-19, contact tracing and quarantine measures, in addition to physical distancing within the community, have proven effective in reducing case numbers.[2] Due to the high sensitivity and specificity in symptomatic individuals, detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection by reverse transcriptase polymerase chain reaction (RT-PCR) is the gold standard method for confirming cases of COVID-19.[3] In contrast, serological assays have lower utility in the initial investigation of suspected cases, but are essential in the development and evaluation of therapeutic agents and to inform modelling and public health policy as this pandemic progresses.

As part of initial laboratory responses, Chinese investigators released the viral whole genome sequence in early January 2020, which enabled the rapid development of RT-PCR workflows for the detection of SARS-CoV-2.[4] However, the unprecedented scale of RT-PCR diagnostic testing has placed extraordinary demands on health care and laboratory systems, with both challenges relating to supply chains of reagents and to the workforce resource required to support population-level testing. Since the start of the pandemic, a range of commercially available diagnostic tests has been released, including RT-PCR assays, point-of-care and laboratory-based serological tests. These tests vary both in analytical performance and in their particular utility in the overall public health response to COVID-19.

Performance aspects of serological tests

Following SARS-CoV-2 infection, specific antibodies to different components of this virus are generated. Depending on the antigen target used by the assay, detection of these antibodies (IgM, IgA, IgG or total antibody) may indicate exposure (non-neutralising antibodies) or potential immunity (neutralising antibodies). To date, a range of serological tests for COVID-19 have been developed, each with particular test characteristics (Box 1). Broadly, these serological tests can be divided into tests that (i) can be performed at the point-of-care; (ii) can be performed in routine diagnostic laboratories, and (iii) can only be performed in specialised reference laboratories (Box 1).

The majority of point-of-care and laboratory-based assays have incorporated either the nucleocapsid antigen (N) or part of the spike protein (S), often the S1 region or the receptor binding domain (RBD). The RBD has been shown to correlate well with the production of neutralising antibodies,[5] while some studies have shown N to be immunodominant, producing an earlier or stronger immune response.[6] Most patients with COVID-19 seroconvert by day 10–14 (~ 80%) following the onset of symptoms, with almost 100% seroconversion by day 20.[7] However, comparisons across published studies are challenging due to the different antigens used in assays, differences in the complexity of patient populations, variations in the RT-PCR assays used as the gold standard for determining the sensitivity of serological assays, and often limited data on the timing of sample collection post-COVID-19 symptom onset. Further, it is not clear whether the type and amount of antibody correlate with severity of disease or, more importantly, with immune protection from re-infection. As noted by the World Health Organization, the Australian Public Health Laboratory Network (PHLN) and the Royal College of Pathologists of Australasia (RCPA), a negative result using a serological test does not rule out SARS-CoV-2 infection, particularly in individuals with strong epidemiological risk factors, and both the PHLN and the RCPA note that there is no role for serological point-of-care tests (PoCT) in the acute diagnosis of COVID-19.[8,9]

Point-of-care testing

As some of the first COVID-19 serological assays available, significant publicity accompanied the release of PoCT. PoCT involve detection of anti-SARS-CoV-2 antibodies through binding to immobilised antigens, generally bound to colloidal gold on a test strip (Box 2). The relatively cheap and simple nature of lateral flow assays means that production is suited to scale-up for increased testing capacity. Post-market validation studies have demonstrated variable performance characteristics, often inferior to that reported by manufacturers, with sensitivities for IgG reported in the range of 53–100% for samples collected more than 14 days after symptom onset, and specificities of 91.7–100%.[10,11] Careful test selection and consideration of the clinical utility before application are therefore critical. The National Pathology Accreditation Advisory Council has existing guidelines on the use of PoCT in Australia.[12] These guidelines cover issues such as clinical supervision for performing PoCT, ensuring test quality, staff training and competency for performing PoCT, and appropriate reporting of test results. More recently, this advice has been extended to serological PoCT for COVID-19, with an emphasis on a robust quality framework to support the implementation and deployment of such tests. Of note, in Australia, the supply of self-testing kits (eg, testing at home) for many infectious diseases, including COVID-19, is prohibited under another Therapeutic Goods Administration regulation, the Therapeutic Goods (Excluded Purposes) Specification 2010.[13]

Katherine Bond[1,2]

Eloise Williams[1]

Benjamin P Howden[2]

Deborah A Williamson[1,2] iD

1 Royal Melbourne Hospital, Melbourne Health, Melbourne, VIC.

2 Microbiological Diagnostic Unit Public Health Laboratory, University of Melbourne, Melbourne, VIC.
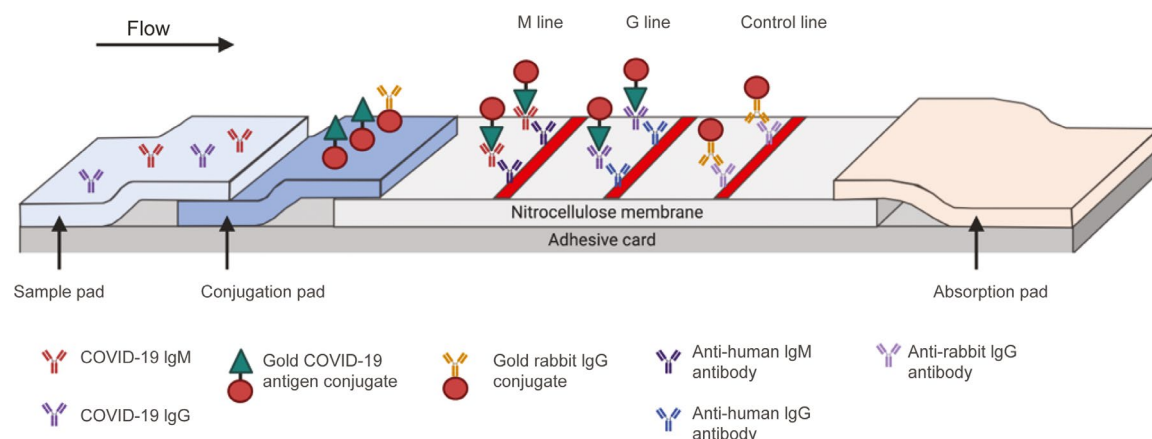
deborah. williamson@ unimelb.edu.au

1 Main serological assays used to date for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

Serological assay	Detection method	Advantages	Disadvantages	Implications
Neutralisation	• Determines ability of test sera to inhibit live virus replication	• Gold standard • Highly specific	• Requires PC3 facilities • Technically demanding • Slow turnaround time • Low throughput	• Only undertaken in specialist laboratories • Gold standard for initial validation of other assays and challenging cases • Not suited to routine testing
Indirect fluorescent antibody (IFA)	• Whole virus inactivated and fixed to a slide • Addition of test sera with fluorescent detection of antibody binding	• Can be undertaken at PC2 facilities once slides prepared • Less technically demanding than neutralisation assays	• Preparation of slides requires PC3 facilities • Less specific than neutralisation • Technically demanding • Subjective end point • Low throughput	• Not available in routine laboratories • Not suited to large-scale testing
Enzyme immunoassay (EIA)	• Recombinant antigen fixed to solid surface (often 96 well plate), test sera applied and antigen–antibody binding detected by enzyme-mediated colour change	• Good sensitivity • Less technically demanding than IFA or neutralisation • Semi-automated • High throughput • Objective end point with machine-based optical density reading	• Less specific than neutralisation • Initial expertise and time required to determine, test and manufacture suitable recombinant antigen	• Generally relies on commercial companies to manufacture and distribute test kits • Suitable for routine testing • Good for screening
Lateral flow EIA	• A particular type of EIA • Recombinant antigen present on immunochromatographic paper, test sera applied to test pad, antigen-antibody binding detected visually by colour change on a membrane	• Variable sensitivity • Least technically demanding • Fast turnaround time for individual tests • Test on demand	• May be less sensitive and specific than laboratory-based assays • Limited scalability • Subjective end point • Data capture less robust	• Suited to point-of-care testing • Can be undertaken by non-laboratory staff • Systems for data capture of results need to be implemented

PC2 = physical containment level 2; PC3 = physical containment level 3. ◆

2 Schematic of a lateral flow immunoassay for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgM and IgG antibodies*

* The sample is added to the sample pad, and then travels by capillary motion to the conjugation pad. Anti-SARS-CoV-2 IgM and/or IgG antibodies in the patient sample then bind to the specific SARS-CoV-2 antigen. This antigen is bound to colloidal gold, which acts as a colorimetric indicator. The bound antigen-antibody-gold complex then travels to the nitrocellulose membrane and bind to specific anti-human IgM or IgG antibodies, with a resultant colorimetric change. To monitor test validity, excess conjugated colloidal gold binds to antibody on the control line, which allows assessment of whether the fluid has successfully migrated across the test strip. Source: Adapted from Li Z, Yi Y, Luo X, et al. Development and clinical application of a rapid IgM–IgG combined antibody test for SARS-CoV-2 infection diagnosis. *J Med Virol* 2020; 92: 1518-1524. ◆

Laboratory assays

A wide variety of laboratory-based serological assays are now available, most commonly enzyme immunosorbent assays (ELISA) or chemiluminescent immunoassay (CLIA/CMIA) format. Assays may be semi-automatic or completely automated, lending themselves to large scale testing and reporting. In general, performance characteristics have been more consistent and closer to that reported by manufacturer's compared with PoCT, with IgG sensitivities in the range of 80–100% for samples collected more than 14 days after symptom onset, and specificity commonly falling between 95% and 100%.[10,11,14]

Use of serological assays

Given the time lag from symptom onset to detectable antibody, serological PoCT have no role in the detection of acute COVID-19. However, there are some settings where serological assays, including PoCT, may have potential utility, including defining antibody prevalence in key populations such as frontline workers and determining the extent of COVID-19 transmission within the community. For other applications, such as identifying individuals for further evaluation of therapeutic immunoglobulin donation and vaccine development and evaluation, assays that assess neutralising antibody response are likely to be required.

Regardless of the type of serological assay used, in order to appropriately deploy serological testing, it is critical to understand the limitations of test performance in the epidemiological context in which tests are used. This is particularly important in a setting such as Australia, where, based on the number of reported cases of COVID-19 (24 236 cases as of 20 August 2020), there is an estimated COVID-19 period prevalence of 0.095% (January to August 2020). As such, even with serological tests that are highly sensitive and specific, the majority of positive tests are likely to represent false positive results. When considering the use of serology to inform policies relating to relaxing of physical distancing interventions, the specificity of the assay becomes critical. If most individuals considered immune actually represent false positive results, then the threshold to maintain immunity (if this indeed correlates with antibody detection) within the community will not be achieved. Consideration should therefore be given for confirmation of initial positive results by either retesting on an assay with an alternative target, or retesting with serological gold standard assays, such as microneutralisation or western blot assays.[15]

Application of serological assays and future research needs

Understanding local transmission dynamics and/ or exposure risk through serological surveys can inform local health policy at an institutional, state or national level. For example, a recent large serological survey in Spain, including more than 50 000 residents, used both PoCT and a laboratory-based CLIA to estimate a seroprevalence across the country of 5.0%, following an initial COVID-19 outbreak in February to April.[16] It was estimated that approximately a third of cases were asymptomatic, while health care workers had a higher seropositivity than the community (10.2% v 5.0%). This is in contrast to health care workers in Belgium, where direct contact with patients with COVID-19 did not increase the odds of being seropositive.[17]

The degree and duration of immunity following SARS-CoV-2 infection is unknown, but if in keeping with other coronaviruses (approximately 40 weeks), immunity is unlikely to be lifelong and may be shorter lived in milder infections.[7] Duration of immunity is a critical area for future research, as it is the key component in models estimating the frequency of SARS-CoV-2 infection incidence in the coming years (eg, second or third waves, or annual seasonal COVID-19 activity similar to influenza), and to determine the utility of policies such as "immunity passports".[18]

Conclusion

The unprecedented demands on laboratories to rapidly upscale testing for COVID-19 has necessarily led to fast-tracking of normally stringent regulatory requirements for test approval, both globally and in Australia. Following the recent publication of peer-reviewed high quality validation data, serological testing is now available in many Australian laboratories. Serological testing will complement the current clinical utility of RT-PCR for SARS-CoV-2 infection diagnosis, highlight local transmission dynamics, and further our understanding of what the future brings for the COVID-19 pandemic.

Competing interests: No relevant disclosures.

Provenance: Not commissioned; externally peer reviewed. ∎

The unedited version of this article was published as a preprint on mja.com.au on 23 June 2020.

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